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Fungus-Growing Ants

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Reprinted from Science, August 5, 1966, Vol. 153, No. 3736, pages 587-604

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A symbiotic relationship exists between an insect and a plant, involving an effective culturing technique.

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The fungus-growers are a New World tribe of myrmicine ants, the Attini, that has developed a unique relation with saprophytic plants. The ants eat only the fungus that they culture, and it is not found outside the ant nest. Many animals feed on fungi, and certain beetles and termites grow them in their nests, but the culturing of fungi as described here is believed to be unique. In this process a flourishing growth of one fungus is produced, and of this fungus only, although the medium on which it grows (the substrate) is suitable for the growth of many other kinds of organisms. When the ants are removed, these other organisms multiply and replace the fungus.

The vital part of the attine nest is the fungus garden. It is the abode of the queen and brood as well as of the fungus. Despite the diversity in morphology of the species, the development and care of the garden are fundamentally similar for all varieties.

Fungus-growing is distinguished from leaf-cutting. All members of this tribe subsist solely, in nature, on the fungus that they culture, but some are leaf-cutters and others are not. The latter pick up vegetal particles of suitable size, or insect excrement, and grow the fungus on these. The leaf-cutters go in files, often on well-formed trails, and cut leaves, flowers, or stems. They are most commonly members of the largest species and belong to the genera *Acromyrmex* and *Atta*. Inconspicuous *Trachymyrmex* and *Sericomyrmex* species may also cut leaves and flowers.

It is the purpose of this article to review the chief features of the life of these ants and of the fungus on which they depend. Because of the economic importance of the large species of *Atta*, and particularly of *Atta sexdens* L. in Brazil, a considerable body of literature has grown up, here summarized, and *A. sexdens* may be taken to represent a high expression of this symbiosis. Studies of other species and genera have made significant contributions to the knowledge of the biological role of the attines and are here reviewed.

Species of this tribe were listed by Linnaeus in 1758, and the type genus *Atta* was named by Fabricius in 1804. Latreille called such ants *Oecodoma* in 1818, and this name was used by the early naturalists, such as Bates, Belt, and Smith in Latin America, for the conspicuous leaf-cutters with soldiers now known as *Atta*. Mayr, from 1862 to 1865, originated the generic names *Cyphomyrmex*, *Apterostigma*, *Sericomyrmex*, and *Acromyrmex*, and he has been the chief contributor to the generic classification. Outlines of the wings, heads, and side views of the ants show differences characteristic of the genera (Figs. 1–4).

The tribe has a wide distribution, from approximately 40° north latitude to 44° south latitude (Fig. 5). The economically important *Atta* species have smaller ranges (Figs. 6 and 7). Their general distribution in South

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American countries is known, although incompletely in Andean areas.

Thomas Belt, who arrived in Nicaragua in 1868, discovered the fungus-growing role of the ants (*1*). He did not realize the full extent of the ant-fungus relationship, thinking that the fungus grew naturally in the damp underground chambers of the ant nest. Belt greatly stimulated Alfred Moeller (*2*), who quoted Belt's conclusions on the frontispiece of his publication.

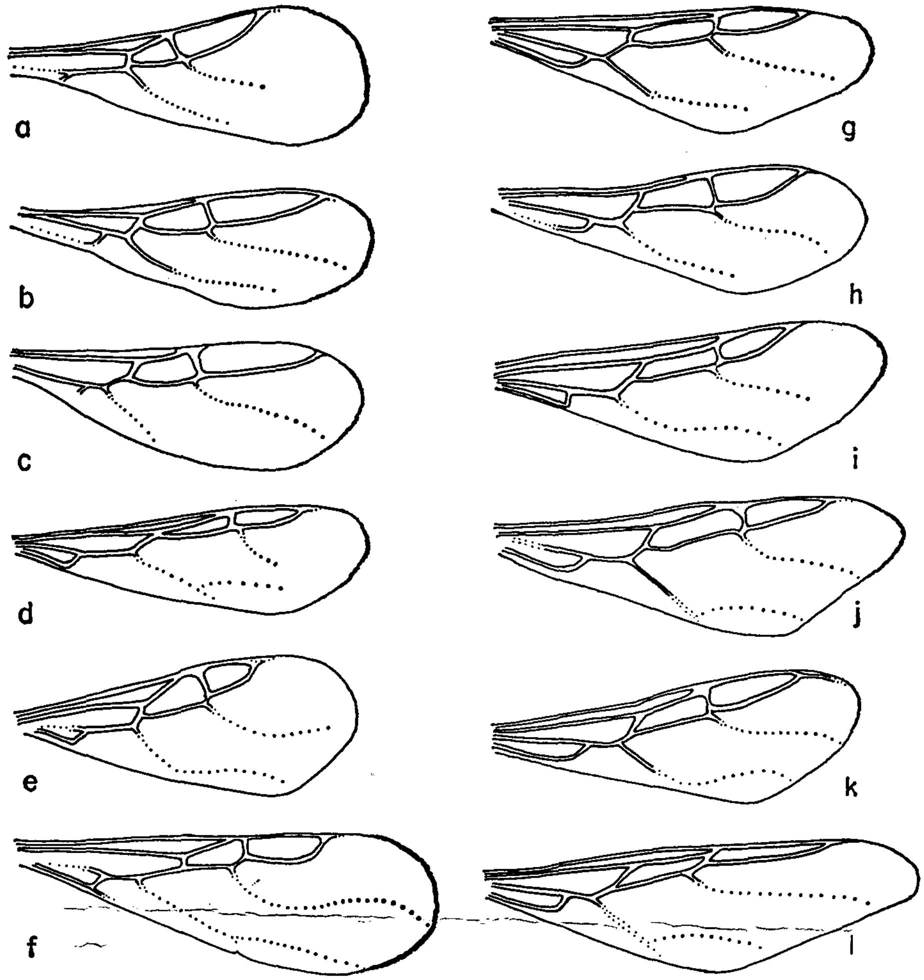
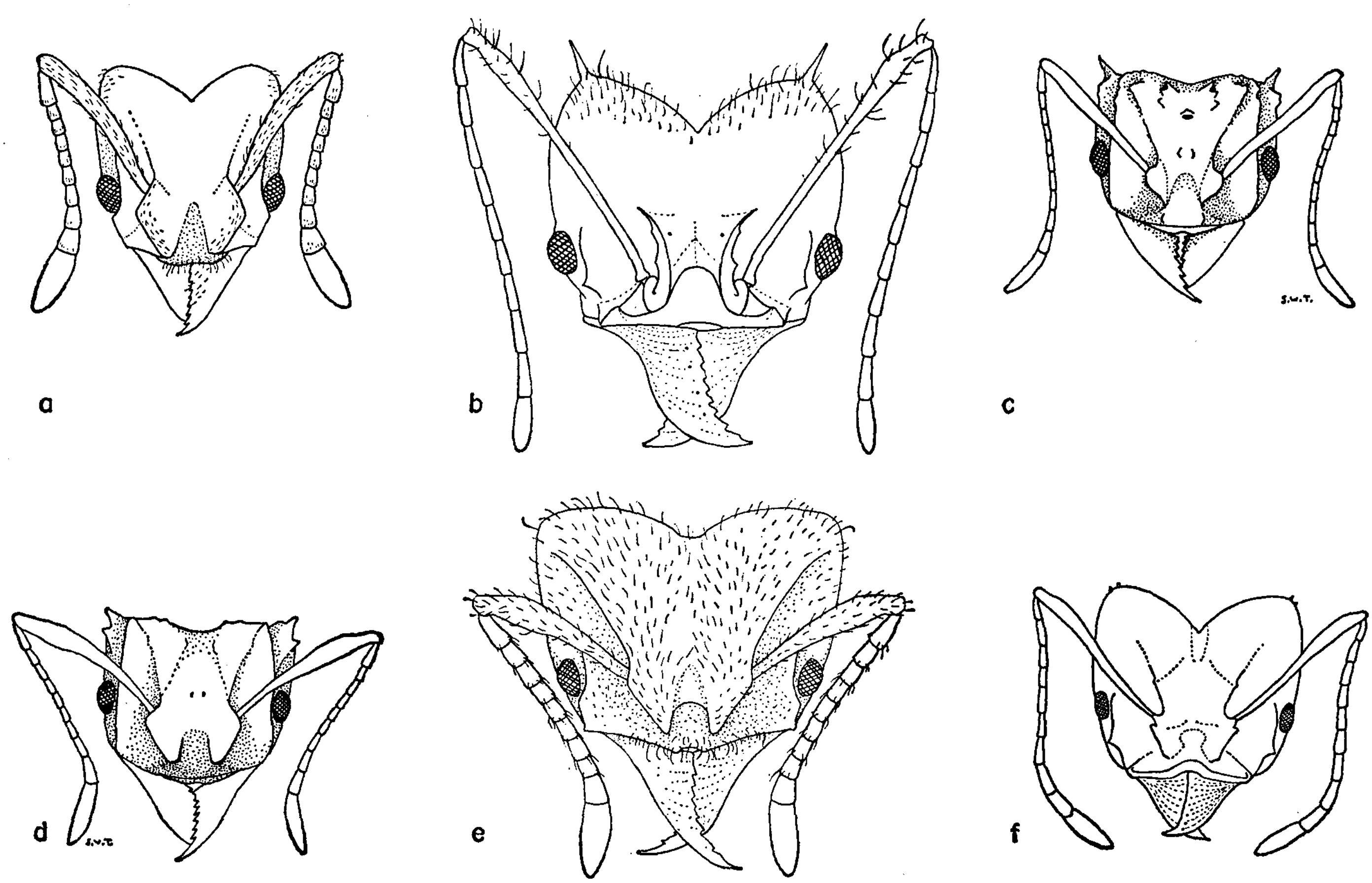


Fig. 1 (left). Wings of representative genera. (*a*) *Cyphomyrmex rimosus Spinola*. (*b*) *C. bigibbosus* Emery. (*c*) *Mycetophylax conformis*. (*d*) *Mycocepurus manni Weber*. (*e*) *Myrmicocrypta buenzlii* Borgmeier. (*f*) *Apterostigma robustum* Emery. (*g*) *Sericomyrmex urichi*. (*h*) *Trachymyrmex cornetzi* Forel. (*i*) *T. urichi* Forel. (*j*) *Acromyrmex (Moellerius) landolti pampanus* Weber. (*k*) *A. (A) octospinosus* Reich. (*l*) *Atta sexdens*.

Fig. 2 (below). Outline of heads of representative species of genera (head length includes mandibles). (*a*) *Mycetophylax conformis* (Mayr); head 0.89 mm. (*b*) *Atta cephalotes opaca* Forel; head 4 mm. (*c*) *Trachymyrmex jamaicensis* E. André; female head 1.8 mm (*d*) *T. jamaicensis*; worker head 1.5 mm. (*e*) *Sericomyrmex urichi* Forel; head 1.5 mm. (*f*) *Acromyrmex (Moellerius) landolti* Forel; head 2.2 mm.



Moeller and then von Ihering, Sampaio, Goeldi, and Huber (*3, 4*) showed the dependence of the ants on the fungus and how this dependence was transmitted from one ant generation to another. Moeller first produced evidence that there could be a fungus sexual or fruiting stage; to this stage he gave the name *Rozites gonglyophora*. His careful mycological studies were basic to later work.

Specimens of *Atta cephalotes* L. and *A. sexdens* had been brought from Guiana to Europe in the 18th century. Their long trails, thronged with workers, had been confused with the files of army ants, *Eciton*, in the popular accounts brought back to Europe from Tropical America. The ants also figured in Central American mythology (*5*).

Almost as soon as the Spanish arrived in the New World they made note of the depredations of ants, which probably were *Acromyrmex* or *Atta*. Bartolome de las Casas in 1559 described the failure of the Spaniards in Hispaniola to grow cassava and citrus trees because of ants whose nests, at the bases of the trees, were "white as snow" (probably the fungus gardens and brood). Article 19 of the cedula proclaimed by the King of Spain on 20 November 1783 for opening Trinidad to immigration states that "the Government was to take the utmost care to prevent the introduction of ants into Trinidad" (see *6*). The ants, of course, had been there all along.

The importance and conspicuousness of *Atta* are attested by the common names which are in general use by the people of various Latin-American countries and used in official publications by their ministries or departments of agriculture (*7*).

Latin-American countries have passed national laws classifying certain ant species as plague animals because of their concentration on economically important plants. For example, Argentina, in Law 4863 of 27 July 1909, considered "*hormigas coloradas*" and "*hormigas negras*" to be plagues. These were later identified as *Atta sexdens* and *Acromyrmex lundi* (Guer.). When the ants are legally classified among the plague animals, the government usually undertakes to carry out extermination methods at the expense of the person occupying or owning the land, if he does not do it himself. The government also surveys yearly the incidence of the animals and the damage done by them and freely disseminates the information. In many countries experts experiment with all control products as they are developed (*8, 9*). A method of controlling *Atta* that is effective in one country will have a calculable probability of success against *Atta* in another country, based on differences in soil, climate, and other variables. No one product has had such complete success in any country that it has replaced all other control methods for very long. People still have difficulty in practicing agriculture in some primitive areas (see *10*).

The Indians of Central and South America have long used the large females of *Atta* as food, and there is no doubt that the gasters filled with eggs have nutritional value. I tried them raw and found them to have a pasty consistency and a bland flavor.

Of even greater importance is the impact of these ants on soil nutrition (*11*). In tropical rain forest areas few animals and few roots of trees go much below the soil surface. In such sites a large *Atta* nest contains far more organic matter, in the form of hundreds of fungus gardens, than any other agency in the soil. This organic matter makes possible the multiplication of great quantities of bacteria, nematodes, insects, and other organisms that can only exist deep underground in such numbers because the ants have carried substrate there.

More pervasive, if less dramatic, is the influence of the nests of smaller and less conspicuous attines. Recent studies of these species in Trinidad showed that there was an average of a nest every 2 square meters in one area (*12*). The size and numbers of the fungus gardens showed that the total impact of these ants on soil nutrition was considerable. They were the only burrowers in the area to construct large underground chambers. These were filled with gardens, as in *Atta*. On the fertile pampas of Argentina the presence of nests of *Acromyrmex* is marked above ground by a richer growth of plants (*11*).

Atta sexdens

The best known and economically most important South American attine is *Atta sexdens* L., together with its subspecies *A. rubropilosa* Forel. This has been the species most commonly studied in Brazil.

Huber (*3*) observed in detail the early stages of colony foundation and watched the female, larvae, and first brood consuming the eggs that she had laid. He estimated that 90 percent of the eggs laid in the approximately 40 to 60 days before the first workers appear were consumed; about 50 eggs were laid daily in this period. Among these Autuori (*13*) clearly distinguished eggs of two kinds, alimentary and reproductive, and he produced an excellent photographic record. The alimentary eggs, laid in the early stages of colony formation, are markedly larger and more globular than the reproductive eggs. The female eats the alimentary eggs herself or feeds them to the first larvae. The internal anatomy of the female gaster (*14*) and the histological basis for the two egg types (*15*) have been described. The spermatheca of the newly fertilized female has been shown to contain some 200 to 300 million sperm as a result of probable fertilization by from three to eight males (*16*).

Autuori has also graphically portrayed the reproductive potential and size of mature nests.

In five successive years the nuptial flights and preparation of the initial nest of the females were noted, then the emergence of the first workers. The average interval between the nuptial flight and the emergence of these workers was 87.2 days, varying from 72.0 days in 1938 to 93.9 days in 1937. Temperatures must have been a significant factor in the time required for development, and Eidmann (*14*) gives these as 18.5° to 25.3°C in the mature garden, according to the depth. The workers used the original nest opening first made by the female.

From examination of a number of young colonies, the average developmental times were found to be as follows: pre-oviposition period, 5 days; incubation, 22 days; larval period, 22 days; pupal period, 10 days.

The appearance of a second nest opening marked the next significant stage. This took place, on the average, 443 days after the appearance of the first opening (minimum 421, maximum 561 days).

The full size range of worker castes appeared between the 4th and 10th month, the soldier caste not appearing until the 22nd month.

The number of entrances to the nests rapidly increased in the second year to 63, 113, and 53, respectively, in three colonies. In the 38th month the numbers had increased to 853, 984, and 1071, respectively. Betan-

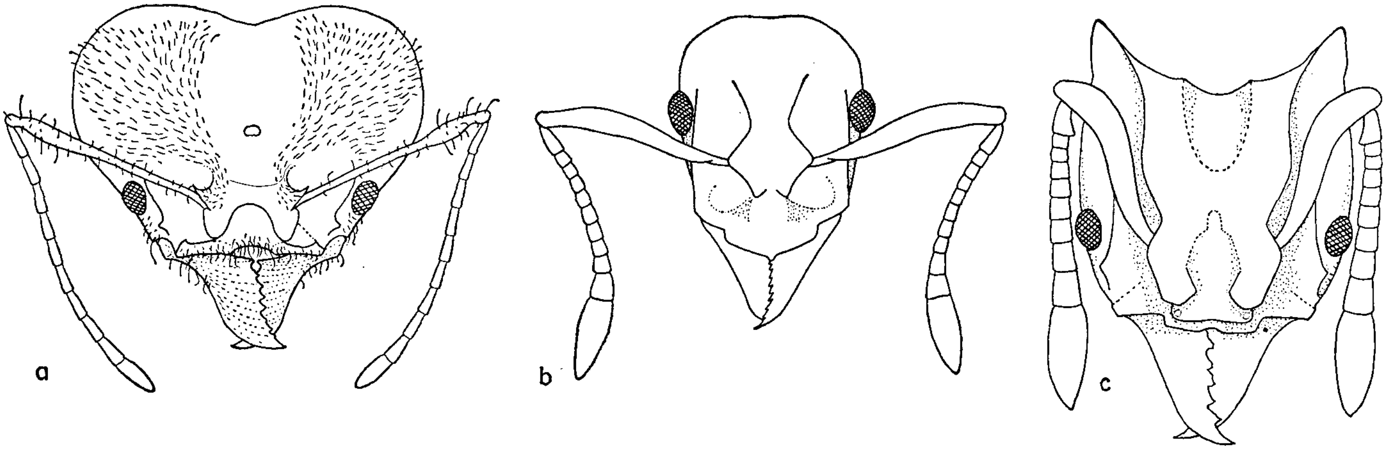


Fig. 3. Outline of heads of representative species of genera (head length includes mandibles). (*a*) *Atta cephalotes opaca*; soldier head 5.8 mm. (*b*) *Apterostigma calverti* Wheeler; head 1.6 mm. (*c*) *Cyphomyrmex bigibbosus* Emery; head 1.3 mm.

court *(17)* used the data in estimating the size of the colony. He maintained that the numbers of openings can be plotted as a logistic curve

$$N = \frac{1000}{271737e - 0.42\ t + 1}$$

where N = monthly average total openings, e = natural logarithmic base, t = number of months from the start of nest building, and 1000 = the theoretical upper asymptote. The maximum size of 1000 openings approximately coincides with the beginning of the production of sexual forms.

A nest of 47 months was opened by Autuori on the day before its second nuptial flight. This nest had 1027 chambers, of which 390 had fungus gardens and ants. There were 38,481 males and 5339 females, a proportion of males to females of 7.2 to 1 [Eidmann (*14*) had given the ratio as 10:1]. An enormous chamber at a depth of 125 centimeters was used as a refuse site and cemetery. It was 90 centimeters high and 120 centimeters in diameter. This contained 1491 adult Coleoptera, 15 adult Diptera, 56 Hemiptera, 40 Mollusca, 4 Reptilia, and 1 Pseudoscorpionida.

Autuori followed one nest for 77 months. It produced a nuptial flight

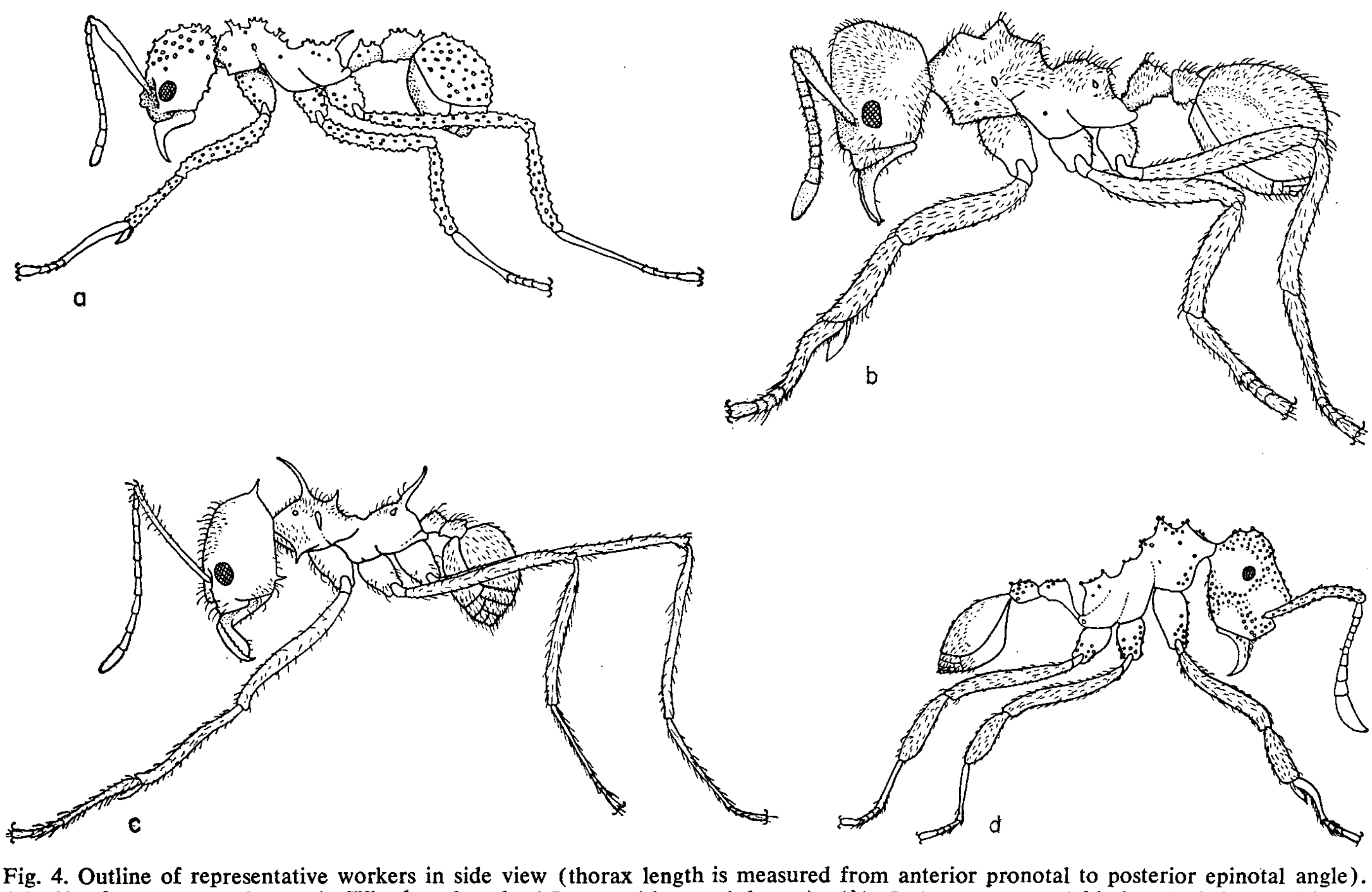


Fig. 4. Outline of representative workers in side view (thorax length is measured from anterior pronotal to posterior epinotal angle). **(*a*) *Trachymyrmex arizonensis* Wheeler; length 4.5 mm (thorax 1.6 mm). (*b*) *Sericomyrmex urichi*; length 3.5 mm (thorax 1.5 mm). (*c*) *Atta cephalotes opaca*; length 7 mm (thorax 2.8 mm). (*d*) *Myrmicocrypta ednaella* Mann; length 2.3 mm (thorax 0.85 mm).**

in its third year and annually thereafter. The loose soil on the surface of the nest was measured before the nest was excavated; it amounted to 22.72 cubic meters and weighed approximately 40,000 kilograms. There were 1920 chambers in this nest, 296 containing refuse, 157 with loose soil, 248 with gardens, and 1219 empty. The empty chambers were believed to have been used for sheltering the sexual forms after they matured and before they left on their nuptial flight. The weights of 184 fungus gardens varied from 15 to 2250 grams, a common weight being 300 grams. It was calculated that, during the life of the colony, 5892 kilograms of vegetation had been used in the nest, with an estimated ratio of 12.4 parts fresh substrate to 1 part discarded substrate.

The mortality of females following their nuptial flight was determined by excavating the nest sites after the resulting colonies had opened the entrance. Autuori concluded that as many as 97.5 percent of the queens died within 100 days. Of the young nests, 99.95 percent were destroyed by natural causes within 15 months. The critical period in colony life was divided into four phases: (i) descent from nuptial flight to ground, 30 to 60 minutes; (ii) excavation of the tunnel and first chamber, 6 to 8 hours; (iii) queen remains in chamber, rearing first brood, 80 to 100 days; (iv) from opening of first entrance to opening of second, 15 months.

Mammals, birds, and other insects, including other genera of ants, were significant predators.

In a later study it was concluded that it was impossible to rid extensive areas of *Atta* colonies. The best that could be expected was the eradication of nests in limited areas that could be thoroughly examined every 3 to 6 months.

Second in importance in Brazil and of primary importance northward to tropical Mexico is *Atta cephalotes*. Where the ranges of the two come together, in Surinam (*18*), British Guiana (*19*), Venezuela (*10*), Peru (*20*), and Panama (*21*), the species show ecological differences, *A. cephalotes* often inhabiting more densely forested areas.

The nests of *Atta cephalotes* are similar to those of *A. sexdens*. There is an extensive system of canals to permit air exchange, with one or more very large chambers (*Abraumgruben*), a meter or more in height, that may be used for refuse. Of 75 fungus gardens found in a mature nest, 44 weighed between 100 and 300 grams (*18*). In appearance, the gardens and the fungus are identical to those of *A. sexdens*. The trails may be well developed (Fig. 8).

Recent studies of young colonies of *A. cephalotes* of known age in Trinidad permit a cataloging of the stages (*12*). These stages show an acceleration in development over those for *A. sexdens* in South Brazil (*22*), due probably to somewhat higher temperatures and higher rainfall. In Trinidad the temperature was 25° to 26°C at the 10- to 50-centimeter depths, under shade, where the young colonies were located (*23*).

Atta cephalotes Nest Stages

Stage 1. The reopening of the initial tunnel made by the female is marked at first by a scattering of soil grains about the entrance, then by the formation of a low tumulus or crater that is often 5 to 8 centimeters in diameter. Trail-making activity starts. Stage duration, 1st and 2nd months (see *10*, plate 2; *22*).

Stage 2. Externally the nest has a small turret of soil grains (10 to 15 centimeters high) and can be distinguished from turrets of other insects and other animals by the large size of the opening. There is a single underground chamber at first, then a second may be started. Only the smallest-to-medium-sized workers are produced. Small trails may extend in all directions. Duration, 2nd to 4th month (see *24*, Figs. 1 and 2; *22*).

Stage 3. The original chimney has now grown to a crater or cone because of the increase in amount of soil excavated. There is still only one entrance. The soil is dumped in the immediate vicinity of the original opening. The two underground chambers are increased to three or four. By this time the colony consists of several thousand workers, some of which are small soldiers. Fewer and more definite trails are formed. Duration, 4th to 7th month.

Stage 4. The number of craters is increased from one to two or three or more. None of these is a turret; they are, rather, low craters of the type many ant species construct, except for their size, 20 to 40 centimeters in diameter. Each crater surrounds an entrance to the nest. Full-size soldiers are being produced. Duration, 7th to 11th month.

Stage 5. A score or two of craters, and a corresponding number of entrances and fungus gardens (similar to those of *A. sexdens*), characterize a fifth stage. Duration, 11th to 16th month.

Stage 6. From now on the colony increases to hundreds of thousands of workers. Sexual broods are produced annually at the appropriate season. Many scores of craters and fungus gardens are developed. It is this mature stage that has been well described in South America and Louisiana (*13, 14, 18, 25, 26*) (Figs. 9 and 10) for several *Atta* species.

The nests of the other species of *Atta* are known chiefly in their mature phases. Those of *A. vollenweideri* and *A. laevigata* in Argentina have been described (*26, 27*). The nest of the Panamanian *A. colombica tonsipes* Santschi is much like that of *A. cephalotes isthmicola* Weber in the same forest (*21*).

General Characters of Attines

Anatomical features that are significant in the life of all fungus-growers include the adult mouthparts, essential to grooming and feeding on the fungus, the infrabuccal pocket that receives dirt from the grooming as well as strands of the fungus, and the pecten or comb used in grooming (*28*; see also *10, 29*) (Fig. 11). Males, females, and workers all use those structures throughout their adult life.

If the ants found in files are strongly polymorphic, they belong to *Acromyrmex* or *Atta*. Characteristically the size range of the worker is continuous, from the smallest, or minima caste, through a series of castes of intermediate size, or media, to the largest, or maxima caste. The extreme in the nonreproductive of *Atta* is usually called the soldier caste and has a disproportionately large head. Such an *Atta* size series (Fig. 12) then would be: (i) minima (total body length, 2 millimeters); (ii) media; (iii) maxima, including soldier caste (total body length, 14 millimeters).

The first two are the only worker castes found in *Atta* nests in the first several months of colony life. A few workers of the maxima sizes are produced during the first 6 months in *Atta cephalotes*, then an occasional soldier will appear (*30*). A large number of soldiers characterizes a mature colony.

The castes of *Atta* and *Acromyrmex* show a division of labor. The minima are largely confined to the fungus gardens and are effective in culturing the fungus and caring for the eggs and small larvae. The minima are so closely attached to the garden that, if a piece of the latter is removed, the minima flatten closely to the irregularities and do not leave the fragment. Workers of the media sizes also tend the gardens and brood, but in addition they cut leaves. The maxima cut leaves and protect the colony. The *Atta* soldiers tend to remain in the garden, often in the vicinity of the queen and brood, and come out mostly when the nest is disturbed. Their mandibles will, with one cut, produce a 5-millimeter cut in human skin; they can cut half-moon sections out of one's leather shoes.

The species of the other attine genera are largely monomorphic. There may be feeble polymorphism in such *Trachymyrmex* as *T. urichi* and *T. septentrionalis.* Laboratory colonies of these, especially of *T. septentrionalis*, may rear progressively smaller workers as the colony deteriorates or, in the latter species, after the height of summer brood raising.

Communication among the members of a colony is accomplished by various means. Studies at the turn of this century indicated that stridulation is an important factor in maintaining cooperation between widely separated ants in underground chambers and that the vibrations are perceived by the ants through the soil (*5*). Recently these vibrations in *Atta cephalotes* have been clearly recorded (*31*).

Tactile communication is indicated whenever two ants of a colony meet. In all species the ants advance with antennae widespread and directed toward the other ant. Then the apices of the antennae meet, and the ants may maintain this position for a second or two, then continue with their previous activities. If the ants are of different species they merely approach without touching antennal apices, then generally act hostile. In these cases the responses may be chemical.

Wilson has shown that the general method of communication is chemical (*32*). The number and complexity of the chemicals are now being effectively studied. The general term for these substances is pheromone, defined as a chemical signal used in communication among members of the same species. He recognizes, among ants generally nine categories of responses: alarm, simple attraction, recruitment, grooming, exchange of oral and anal liquid, exchange of solid food particles, facilitation, recognition, and caste determination. My observations of numerous attine species indicate that, of these nine, the first four and the last three are the usual responses. A further modification of the chemicals would enable the ants to distinguish their own colony mates from other members of the same species.

A special use of pheromones is their use in making a scent trail (*33*). Ants of several attine species have been shown to follow one another by this means. The ants deposit droplets at intervals. These droplets form the trail, and other ants follow. The trail, in nature, may be invisible to the human eye, as reported for *Trachymyrmex isthmicus* (*21*). An alarm pheromone in mandibular glands of *Atta sexdens rubropilosa* has been isolated (*34*).

Visual means of communication have been little studied. Workers and soldiers of *Atta* will respond to a waving of the finger on the outside of the glass or plastic tube in which they are confined. Both males and females, especially males, have large eyes, and it seems possible that it may be a combination of chemical, auditory, and visual stimuli which brings the sexes together in the nuptial flight.

The Brood

The attine brood is normally covered by the mycelium of the garden, contrary to the impression given by photographs of the brood in the early

Fig. 5. Distribution of *Attini.*

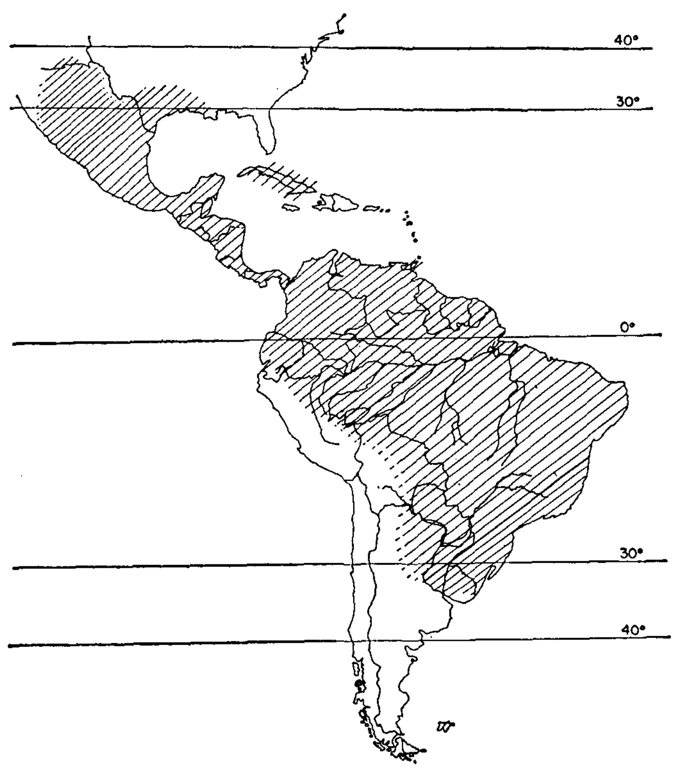


Fig. 6. Distribution of *Atta.*

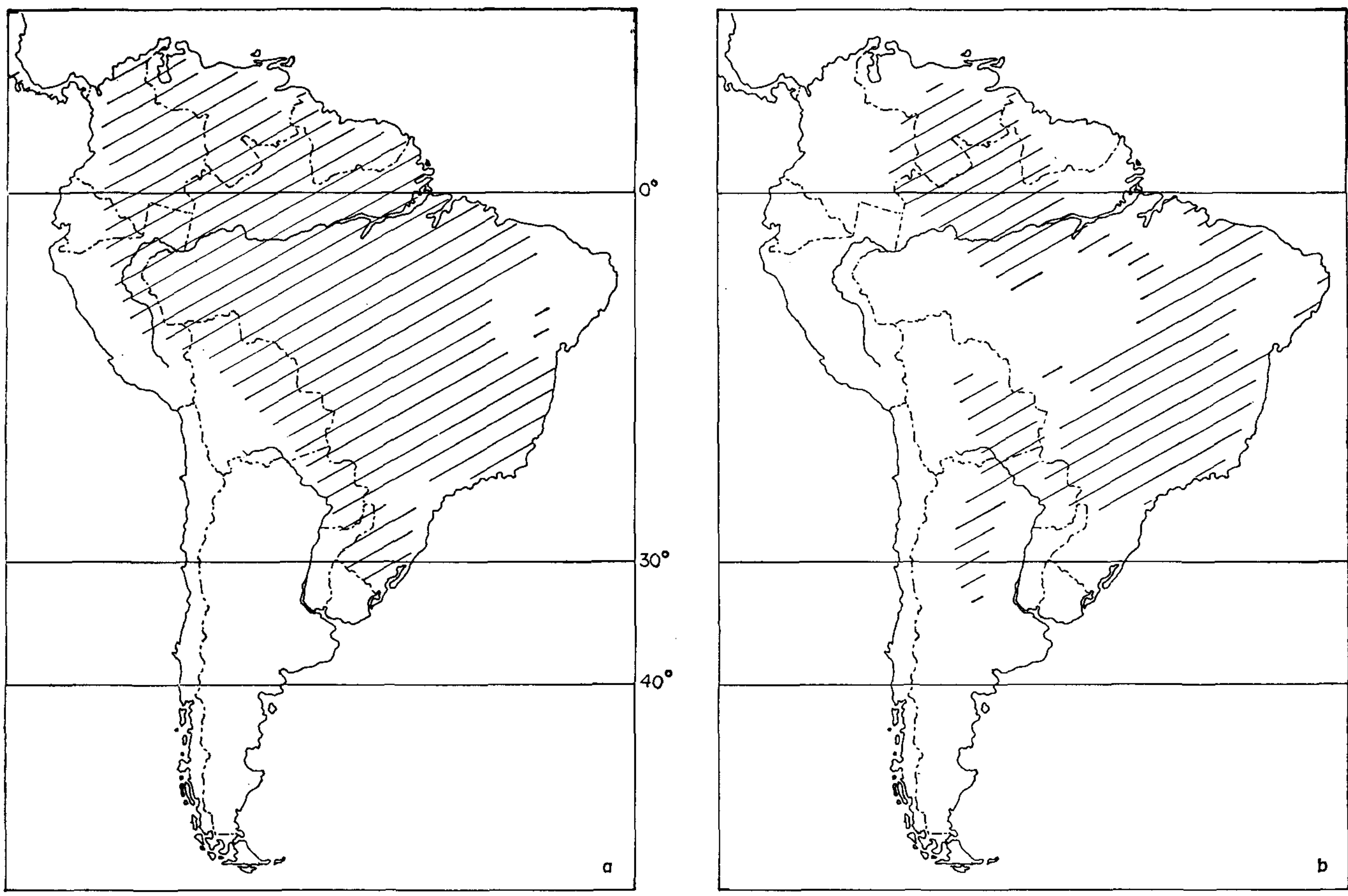


Fig. 7. Distribution of four species of *Atta* in South America [Brazilian distribution after Gonçalves (*9*)]. (*a*) *A. sexdens* L. The species is also found in the western and drier part of Panama. (*b*) *A. laevigata* F. Smith, a species found in the drier part of South America. (*c*) *A. vollenweideri* Forel, a species confined to southcentral parts of the continent. (*d*) *A. cephalotes* L. The species also extends through Central America into Mexico.

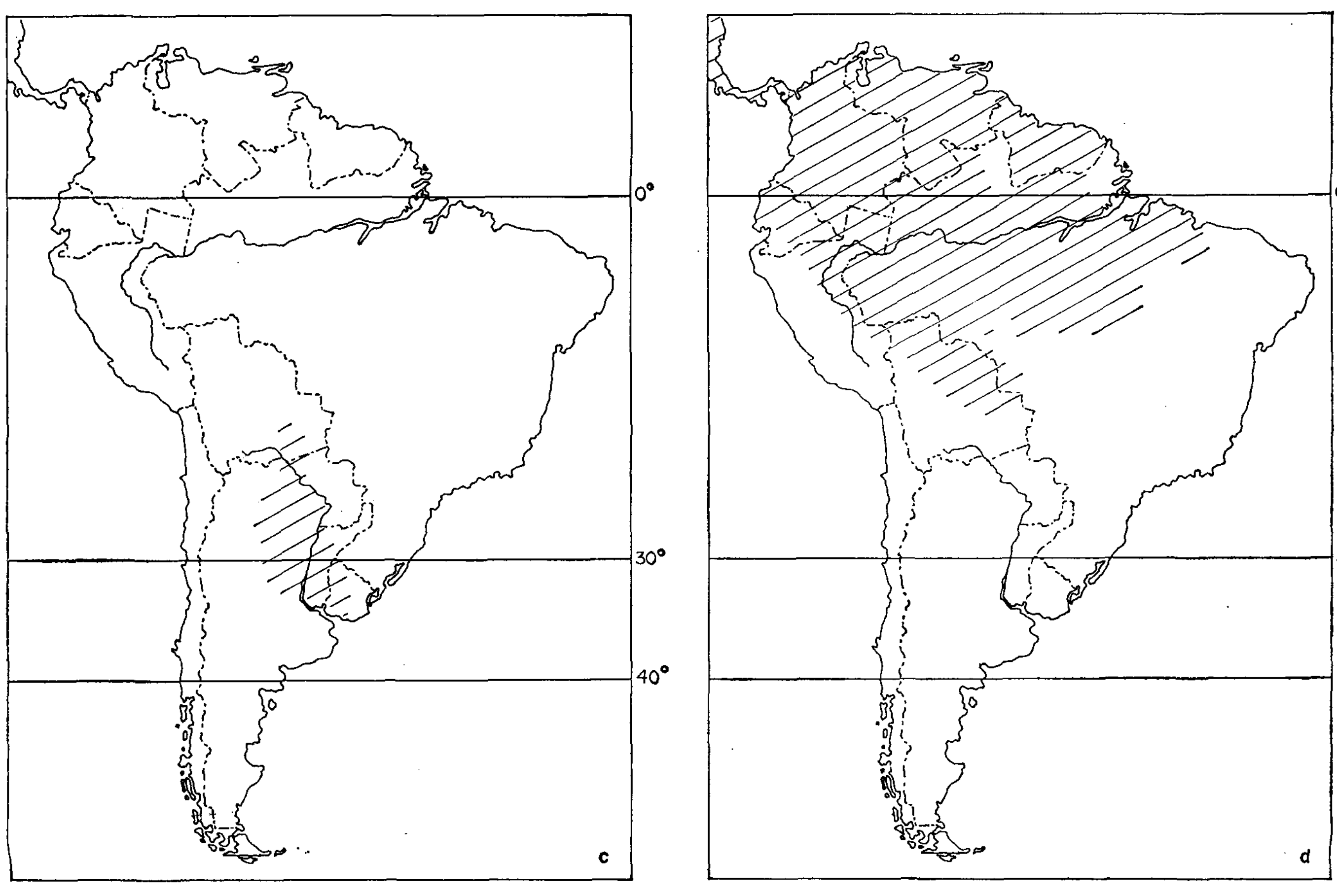


Fig. 8 (left). Forking trail made by a large colony of *Atta cephalotes* coming from the foreground and extending into areas where leaves were being cut. Parts of the trail were 30 centimeters wide. Trinidad.

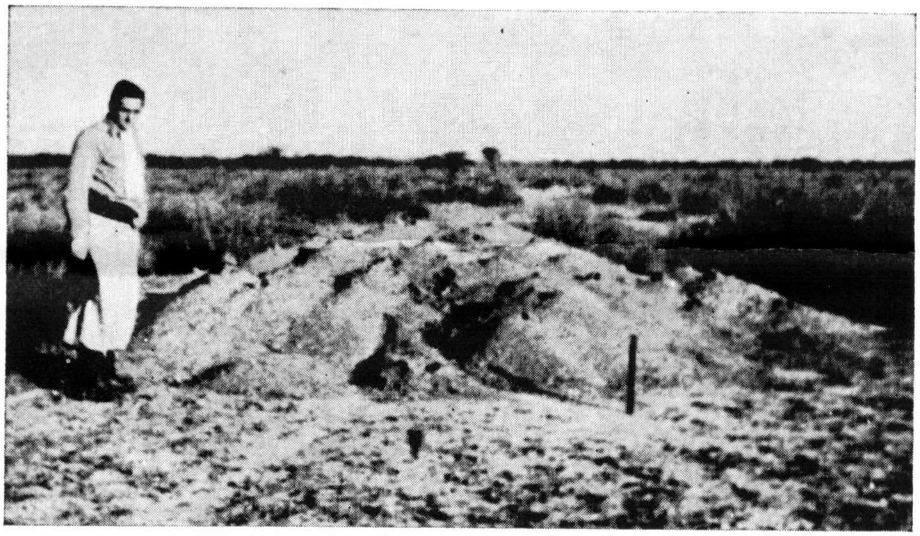


Fig. 9 (center). Nest of *Atta vollenweideri* at the southern margin of the distribution of *Atta*, Santa Fe Province, Argentina. [Photograph by Bonetto]

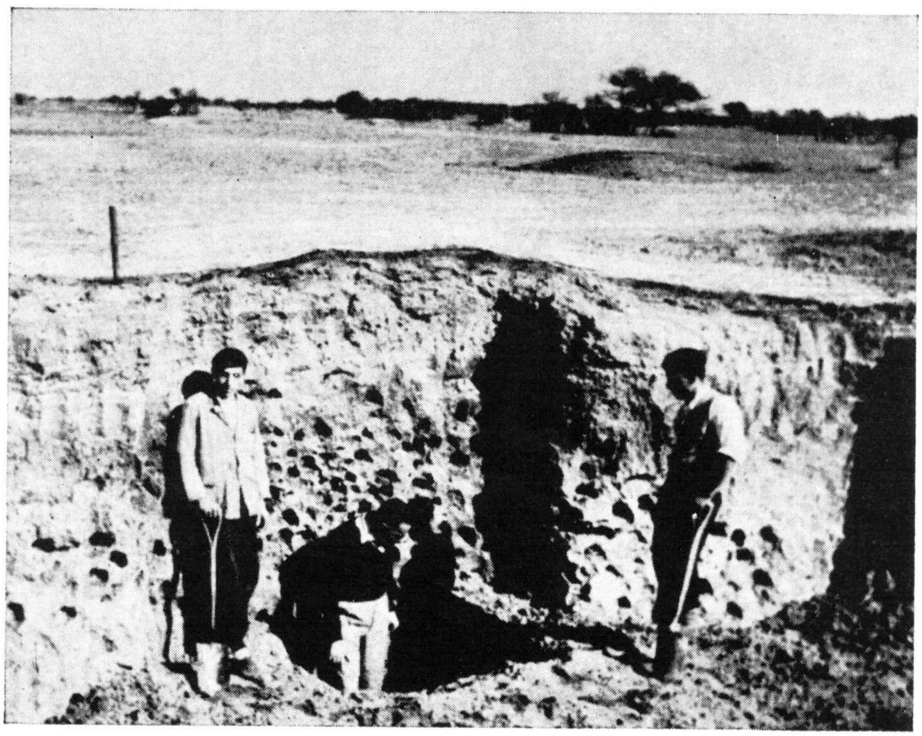


Fig. 10 (bottom). A nest of *Atta vollenweideri* in cross section. [Photograph by Bonetto]

stages of *Atta* colony formation (*13*). In these early stages the garden has not yet attained its luxuriant hyphal development. The eggs are especially difficult to distinguish in their mycelial mesh. The *Atta* queen lays eggs unassisted at first; after the workers mature, they remove the eggs as they appear at the cloaca. The female of the more primitive species like *Myrmicocrypta buenzlii* lays eggs unassisted during the entire life of the colony.

Soon after the larvae emerge they rotate so that the mouthparts are uppermost. For the duration of its life the larva lies on its side or dorsal surface, so that the head capsule is exposed. When the mouthparts are protruded ("pouting"), the nursemaid workers respond by placing a cluster (staphyla or "kohlrabi" of older authors) (Fig. 13, *b*, *d*, *g*) of inflated hyphae or a tuft of ordinary hyphae on them. Slender hairs posterior to the head capsule on the ventral surface of the body help to hold the fungus in place. The larval mandibles are minutely spinulose and puncture the fungal cells.

The distension of the body and increasing opacity of the skin signifies the semipupal stage. The *Atta* semipupa is bean-shaped, and the appendages may be faintly seen through the larval skin (Fig. 14). The pupa (Fig. 15) is naked, with segments and appendages clearly outlined. The compound eyes darken early, then general pigmentation proceeds in a sequence that is useful for determining ages.

The pupa later starts moving its appendages. Anterior legs and antennae are the first to move slightly, and there are slight body tremors. The mouthparts, which are extended throughout pupal life, start moving. Meanwhile the workers have been in attendance, licking the skin intently. The ant cannot emerge from the pupa without this assistance, although it can start to emerge anteriorly from the exuviae.

The callow stage of the adult is reached when the ant is out of the exuviae and can stand unsteadily on its legs. It is markedly paler than the mature ant, and the legs are pale brownish yellow in all castes in various species. The workers continue to groom it and assiduously lick the wings of the young male or female. The callow can feed by itself, taking a staphyla or a few strands of the fungus between the mandibles.

The highly developed social nature of the attine colony is well shown by this brood care and complete depend-

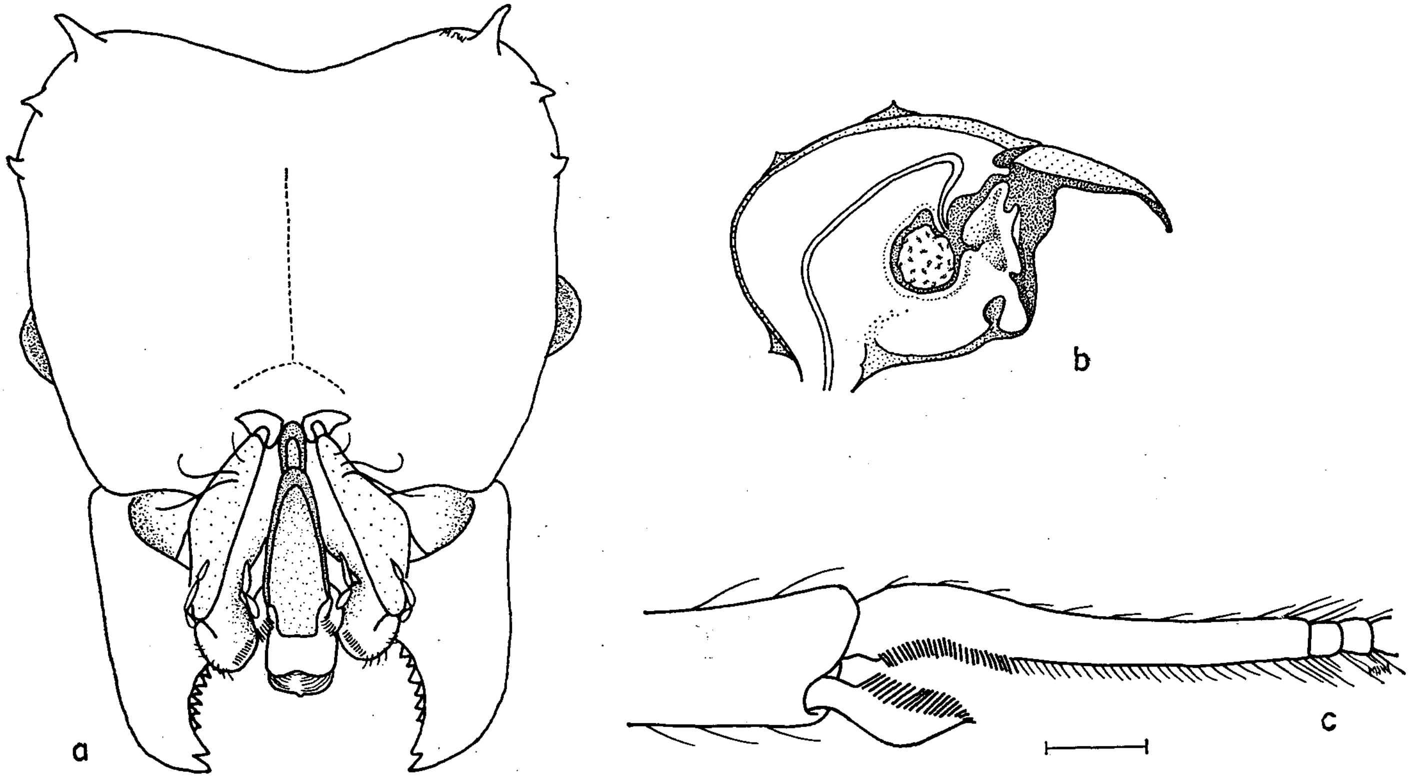


Fig. 11. Significant anatomical features. (*a*) Mouthparts of *Acromyrmex lundi* (Guérin), from behind, showing the extended central tongue or hypopharynx. (*b*) Sagittal section of *Atta* head, showing the infrabuccal pocket in the rear of the mouth. It contains a fungal mass that will be the nucleus for the new garden when the female leaves the parental nest [after Huber]. (*c*) Part of the foreleg of an *Atta* worker, showing the pecten used for cleaning the appendages. The bar represents 0.25 millimeter.

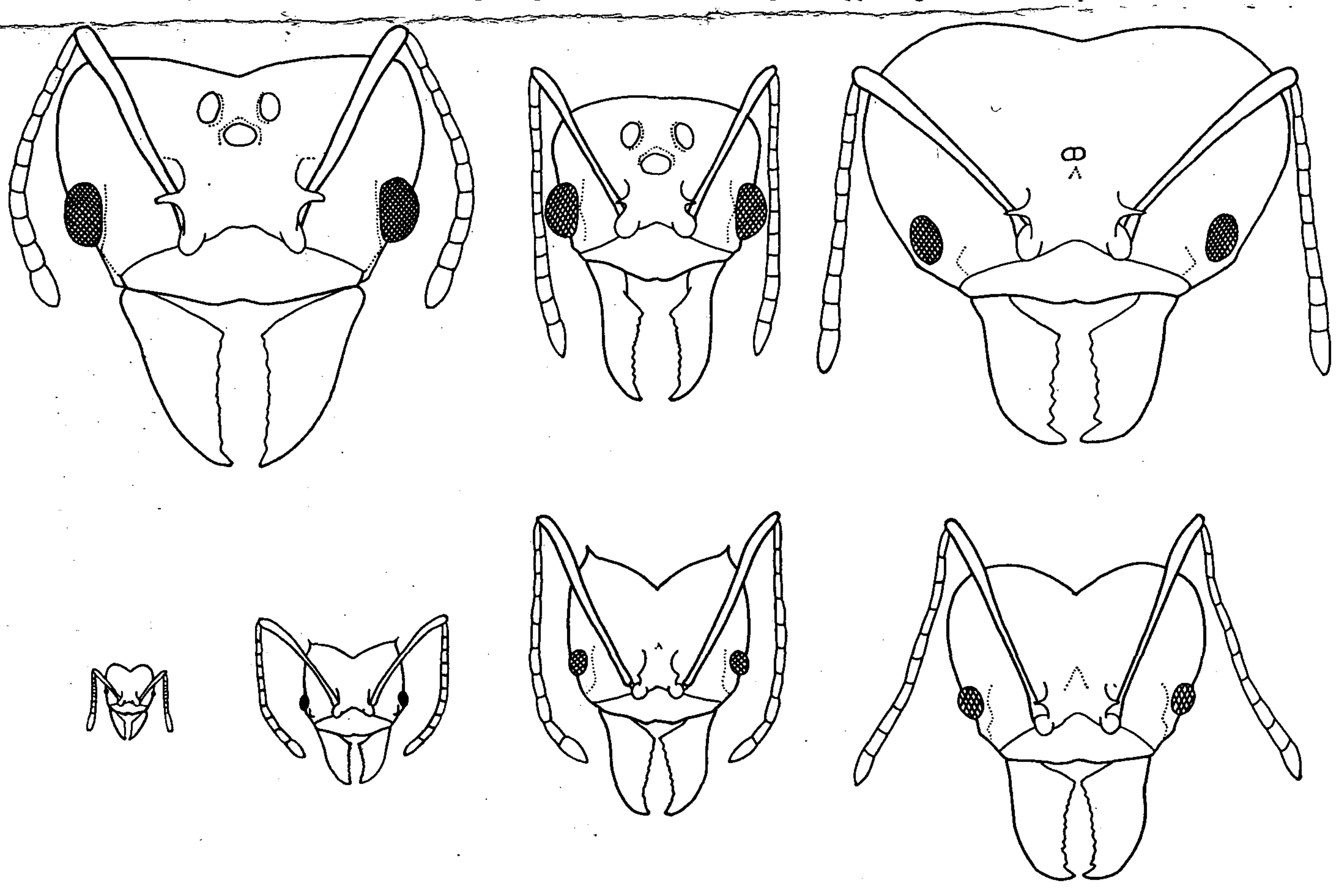
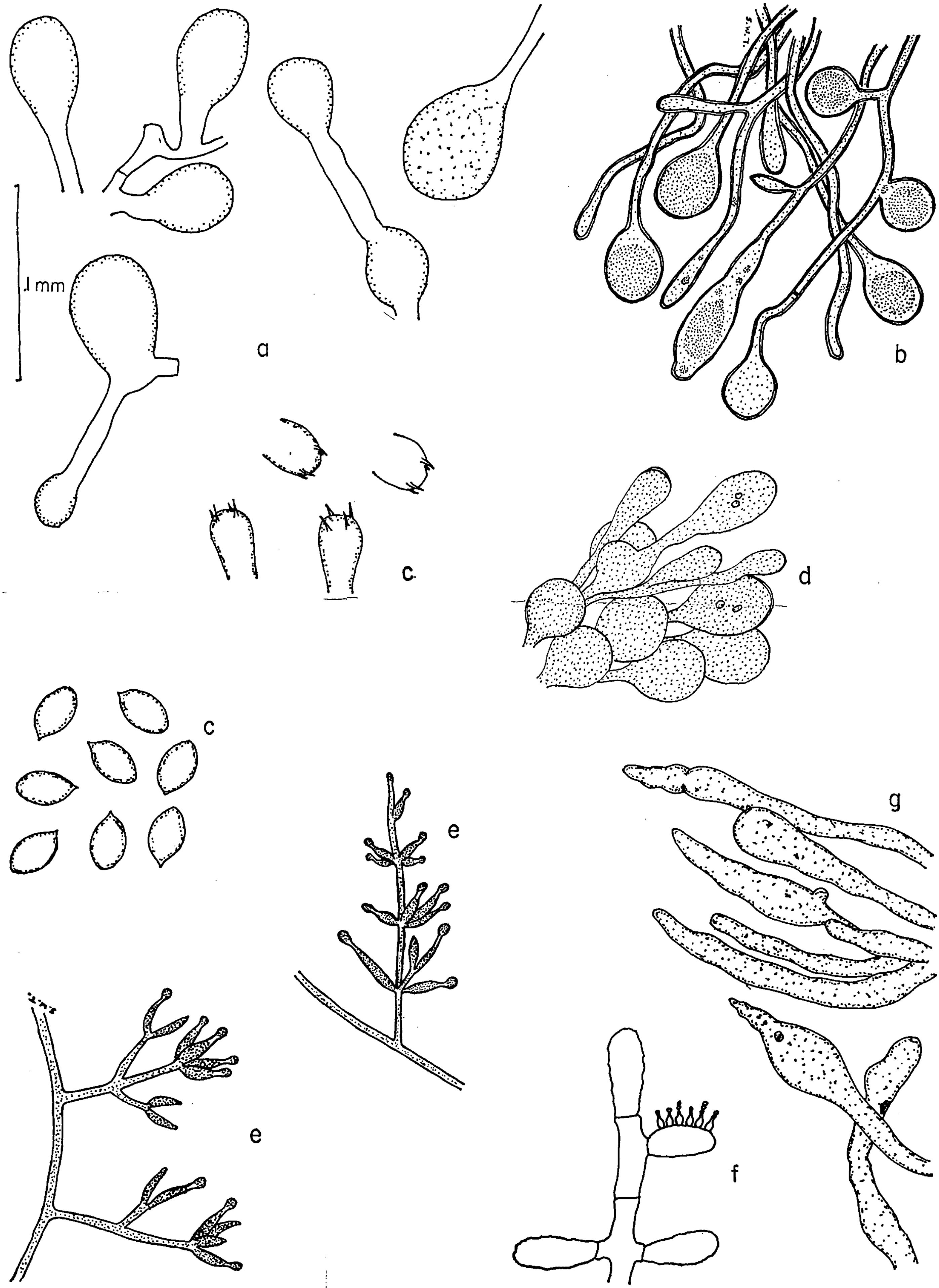


Fig. 12. *Atta cephalotes*. Outline of heads of castes, to scale. Lengths include the mandibles; width between eyes, in millimeters, given in parenthesis. (Top row, left to right) Female, 5.8 mm (3.92); male, 4.1 mm (2.28); soldier, 5.8 mm (3.54). (*Bottom* row left to right) Minima worker, 0.9 mm (0.58); media worker, 1.8 mm (1.16); media worker, 3.5 mm (2.03); maxima worker, 4.3 mm (2.53).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ence of the larvae and adults on the fungus.

Indispensable to studies of the brood and to many other attine studies is some type of observation nest in which the ants can live normally and still be examined under the microscope. A type used for many species is shown in Fig. 16.

Other Colony Characters

Most of the year the fungus-growing ants have adequate food, thus insufficiency of food is not one of the hazards they face. But these ants are subject to predation, parasitism (as from mites and phorid flies), and such other ordinary dangers as accidental injury. Workers of the larger species that go long distances in files may become lost. They may drop from trees into unfavorable sites or be diverted away from their trail. Individual workers are known to have lived 19 months in the laboratory (*29*). The males usually live only a few weeks after maturity, dying the day of their nuptial flight, while fertilized females (which are easily distinguished) are perhaps the longest-living adult insects. Brazilian authorities believe 20 years may be a possible life span for the *Atta* queen (*35*), and a female *Trachymyrmex zeteki* is known to have lived more than 5 years (*36*). Individual workers marked with colored lacquer have lived in the Swarthmore laboratory for between 1 and 2 years. These are *Myrmicocrypta buenzlii* and *Trachymyrmex septentrionalis*; two living females of the latter species are now 4 years old.

The size of the colony has a bearing on the size of the garden or gardens that can be cultivated. A score of workers, or a few score, cannot maintain a garden larger than a few cubic centimeters because of inability to give all parts the requisite care. Colonies with the smallest populations appear to be those of certain *Cyphomyrex* and *Apterosigma* species, with 100 or 200 workers (*21, 37*). Representative colonies of three Trinidad species of other genera were far larger. One of *Myrmicocrypta buenzlii*, with a single garden 8.5 by 7.5 by 5.0 centimeters, had 1558 workers. One of *Sericomyrmex urichi*, with seven gardens varying from 3.4 by 7.0 by 1.0 to 11.5 by 8.0 by 5.0 centimeters had 1691 workers. One of *Trachymyrmex urichi*, with six gardens varying from 4.5 by 3.0 by 3.0 to 8.5 by 11.4 by 12.0 centimeters, had 763 counted workers, and those missed in the count brought the estimated total to 1000 (*11*). An *Acromyrmex octospinosus* colony had 7160 workers (*38*).

The Nest and Garden

The nest is large and conspicuous only in *Atta*. In some species of *Acromyrmex* a large colony may be hidden under what appears to be a pile of trash at the base of trees (*19, 38*). Other species of *Acromyrmex* may form a turret constructed of soil and grass sections (Fig. 17) or a dome of thatch (Fig. 18), beneath which are the garden or gardens.

The external indication of nests of species of other genera may be a low, semicircular crater (Fig. 19) or a circular crater (Fig. 20), which is frequently blown away in windy sites, where the ants habitually nest. Other types of nests and entrances have been sketched or photographed (see *10, 19, 21, 37*). In many cases the type of nest structure is a valid species character based on behavior.

The largest gardens are those of the genera of largest ants, *Acromyrmex* (Fig. 21) and *Atta*. The former may have a single very large garden (40 to 50 centimeters in diameter), or it may have a number of smaller gardens (25 to 30 centimeters). Those of *Atta* appear to be more consistently globular and some 20 to 30 centimeters in diameter.

The gardens of *Trachymyrmex* tend to have a laminated structure, the septa being hung from rootlets entering the ceiling of the chamber. Some gardens rest completely on stones on the floor of the excavation.

Gardens of *Sericomyrmex* tend to be disproportionately large. In *S. amabalis* and *S. urichi* the substrate is often pale brown and fruity.

The gardens of *Cyphomyrmex*, other than *C. rimosus*, are small (20 to 50 millimeters in diameter), corresponding to the size of the ant. Those of

Fig. 13 (facing page). Forms of ant fungi. (*a*) Fungus of *Atta colombica tonsipes* Santschi, showing inflated hyphae from an artificial culture in a flask of sterile soil. (*b*) Fungus of *Trachymyrmex jamaicensis* E. André, showing typical staphyla from a fresh fungus garden. The hyphae have been teased apart. The inflations are characteristic of the fungi of the higher attines and are 30 to 50 microns in diameter. (*c*) Fungus of *Cyphomyrmex costatus* Mann which attained the sporophore stage and has been identified as a new species of *Lepiota*. The spores are 5 by 8 microns, and the basidia are 10 microns thick. (*d*) Fungus of *Trachymyrmex septentrionalis* McCook. A fresh staphyla. (*e*) Conidial form of fungus found under certain conditions in the garden of *Trachymyrmex septentrionalis*. The ants immediately leave such gardens. (*f*) A conidiophore of the fungus found in another colony of the same ant under abnormal circumstances. (*g*) Fungus of *Atta cephalotes ithmicola* Weber, showing staphyla forming in an artificial culture on Sabouraud's dextrose agar. The thickest hypha is 39 microns in diameter.

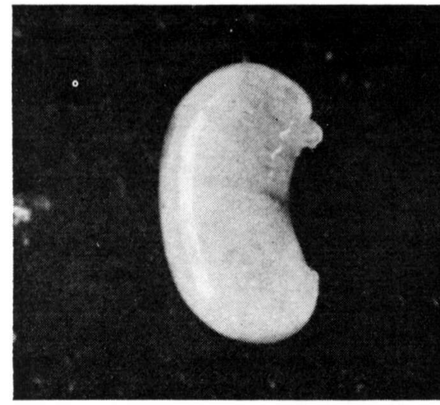


Fig. 14. Semipupa of a large worker of *Atta cephalotes* faintly showing ventral appendages under the taut larval skin. The head capsule is at upper right, the anal hillock at lower right. About 7 millimeters long. Trinidad.

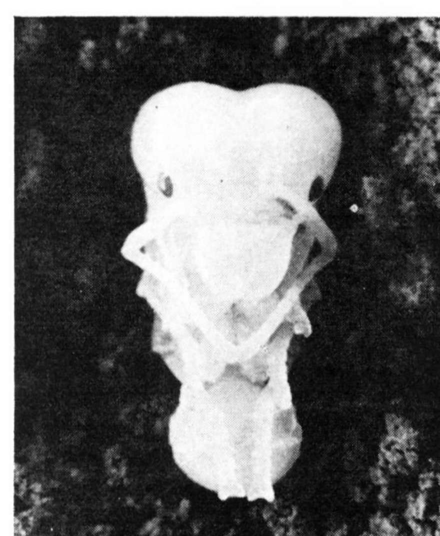
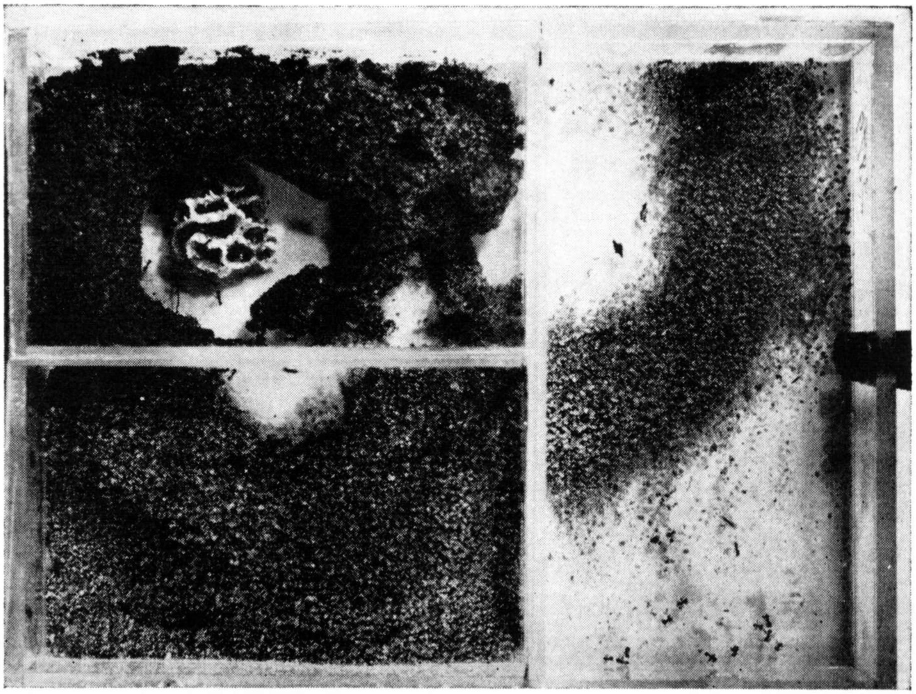


Fig. 15. Pupa of the soldier caste of *Atta cephalotes*. Legs and antennae are folded ventrally. The white skin and beginning pigmentation of the eyes show that this pupal form is a few days old; 12 millimeters long. Trinidad.

Mycocepurus smithi Forel are approximately 20 to 25 millimeters in diameter. The gardens of the small ants of *Myrmicocrypta buenzlii* Borgmeier (Fig. 22) are often 50 to 80 millimeters in diameter. All three genera have gardens with cells a few millimeters in diameter. The gardens consist of fragments of vegetal matter and of the antfungal mycelium. Sometimes carcasses of insects are incorporated as substrate. Certain *Apterostigma* gardens may be enclosed in a mycelial veil (Figs. 23 and 24).

The most fragile gardens are those of species like *Mycetophylax conformis* (Fig. 25) and *Acromyrmex (Moellerius) landolti* when the ants use grass for substrate. The pieces are relatively long and narrow, creating an irregular mesh of cells, and the mycelium is scanty. The weight of fresh leafy substrate necessary to build up one fungus garden (about 1200 cubic centimeters, weighing approximately 130 grams) in *Atta cephalotes* has been determined (*39*). In a young, vigorous colony 670 grams of substrate was used, in the course of 9 months, before any was cast out. The second garden was built in the next 3 months, when a total of 2060 grams for the two gardens had been taken in. It may be expected that a mature colony will use, in nature, fully 1 kilogram of fresh leaves for a garden of this size. Many gardens in nature have twice this bulk and must take fully 2 kilograms.

Among the interrelated factors involved in maintaining the fungus garden are behavioral, fungistatic, bacte-



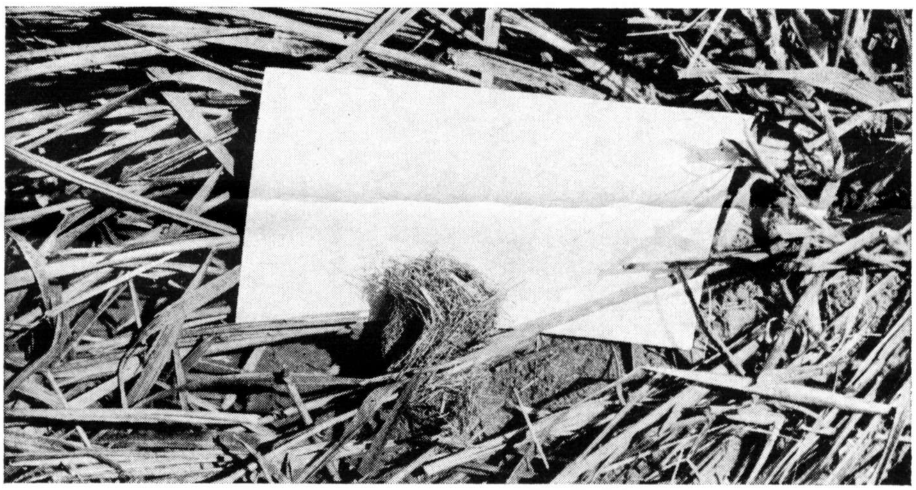


Fig. 16 (top). A small colony of *Trachymyrmex septentrionalis* in standard Plexiglas observation nest. Each of the three chambers is connected to the others by a tunnel. This colony has cast out sand in the form of a semicircular crater, as it would have in nature. Soil is always removed from the vicinity of the fungus garden. Inside dimensions, 205 by 152 by 18 millimeters.

Fig. 17 (center). External indication of the nest of *Acromyrmex (Moellerius) landolti* Forel in the form of a turret, characteristic of the species, of soil and grass stems (*Trachypogon plumosus*). Such a turret, on the llanos of Venezuela, tends to reduce evaporation from the underground gardens constructed on the same grass. Card, 76 by 127 millimeters.

Fig. 18 (bottom). Mound nest of *Acromyrmex (Moellerius) heyeri* Forel. Thatch covers the single chamber, which contains one large fungus garden. The 15-centimeter ruler is covered with ants. Uruguay.

riostatic, and, perhaps, growth promoting factors. These are promising lines of study currently being investigated and not hitherto fully appreciated. The evidence so far is mostly suggestive, and the need for experimentation is obvious.

The early investigators of *Atta* described the behavior of the new queen and first broods in caring for the fungus (*3, 4*). The female feels the fungus almost continually with her antennae and deposits anal droplets on the garden. This behavior has been shown to be universal among the attine workers (*40*).

If the ants are leaf-cutters the procedure is as follows: The cut leaf sections are brought into the nest. These sections may be placed at the base of the garden, or they may be brought to the sides and top. Next comes the cutting into much smaller pieces, a millimeter or two in diameter. Before or during this process the pieces are licked all over. The ants then press along the edges of the pieces with the sharp mandibles so that they become wet and pulpy. An ant may take the piece and place it under its body, curve the tip of the abdomen (gaster) forward, and deposit a clear anal droplet. The leaf piece is then inserted into the garden. The ant uses its forelegs with a side-to-side motion and forces the piece into place with the aid of the mandibles. An ant then picks up tufts of the fungal mycelium and places them at intervals on the prepared substrate (*41*). The hyphae grow out in all directions from the tuft.

If the ants are not leaf-cutters, the procedure may differ only in preliminary details (*29*). Caterpillar excre-

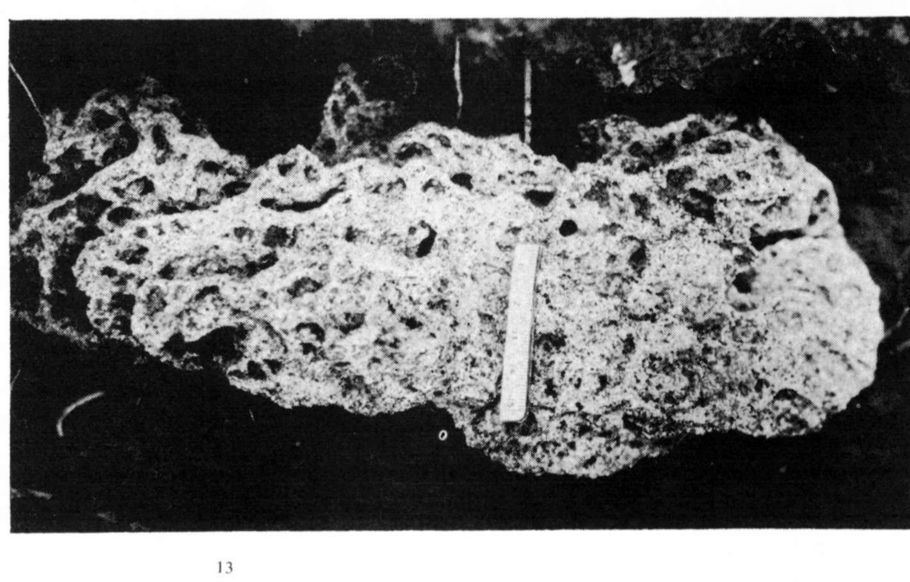
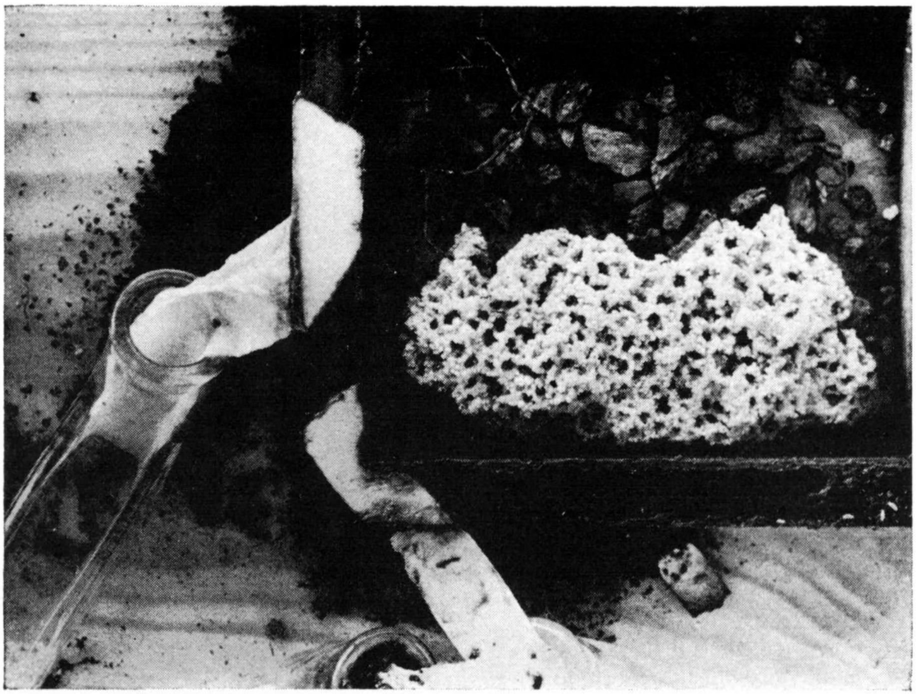
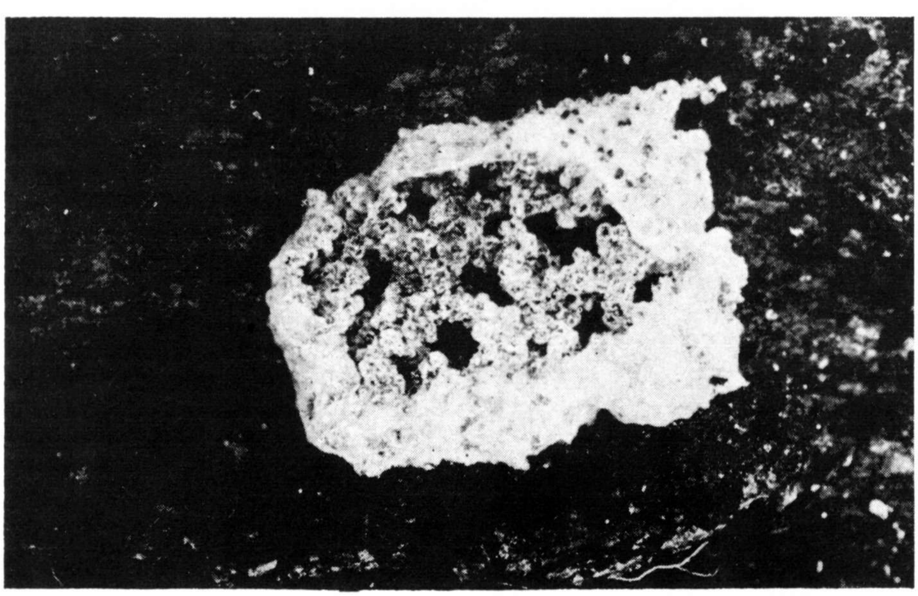


Fig. 19 (top). External indication of the nest of *Trachymyrmex septentrionalis* in the form of a lunate crater characteristic of the species. The single entrance is below the middle of the 15-centimeter ruler. Florida.

Fig. 20 (center). Crater entrance, characteristic of the genus, to the nest of *Mycetophylax conformis* on the coast of Trinidad. Crater, 7 centimeters in diameter. Constant trade winds tended to blow away the excavated soil, and showers in the rainy season washed it away, but the ants repeated this pattern of crater formation.

Fig. 21 (bottom). Fungus garden of *Acromyrmex octospinosus* shown with a centimeter ruler. The garden was about 31 centimeters long and 11.5 centimeters high and was under a 3-meter log in a cavity in sand. Trinidad.





ment may be broken into the original leaf sections, which are then planted in the garden. Woody particles or other vegetal debris and white cassava starch granules may be dragged to the garden, defecated on, then planted with the mycelium. I have dyed individual cassava particles with neutral red or nigrosine black in order to follow their history in the garden. Small attines may drag parts of insect carcasses to the nest and scatter them over the garden (*19, 21, 29*). These, too, acquire islands of mycelial growth.

All attines tend the garden similarly. The ants walk over all exposed parts and down into the cells, using the apices of the antennae as probes to determine the condition of the fungus. They lick the hyphae and occasionally eat them. It appears that droplets are normally deposited only on the garden. A garden fragment placed, with ants, on a nutrient agar plate will be maintained despite surrounding contaminants (Fig. 26).

A fungistatic factor is indicated by the fact that only one recognizable fungus is present in the normal garden. The older assumption that other fungi are weeded out may be valid only to a limited extent (*40, 42*). What is true is that spores of other fungi are brought into the garden on substrate but do not proliferate.

A selective bacteriostatic factor, like a fungistatic factor, is indicated by the control of bacterial growth. Some types of growth may normally be present, others may be harmful. When the ants are removed (*18, 29, 40*), the garden quickly degenerates, either becoming a slimy mass from bacterial action or becoming overgrown with sporulating alien fungi.

A growth-promoting factor is indicated by the growth of the ant fungus

Fig. 22 (top). Fungus garden of *Myrmicocrypta buenzlii* in an observation nest of the type used in 1934–35 in Trinidad. This consists of a wood frame 2.5 centimeters high, with glass top and bottom. Needed moisture is brought in from the bottle by a paper wick.

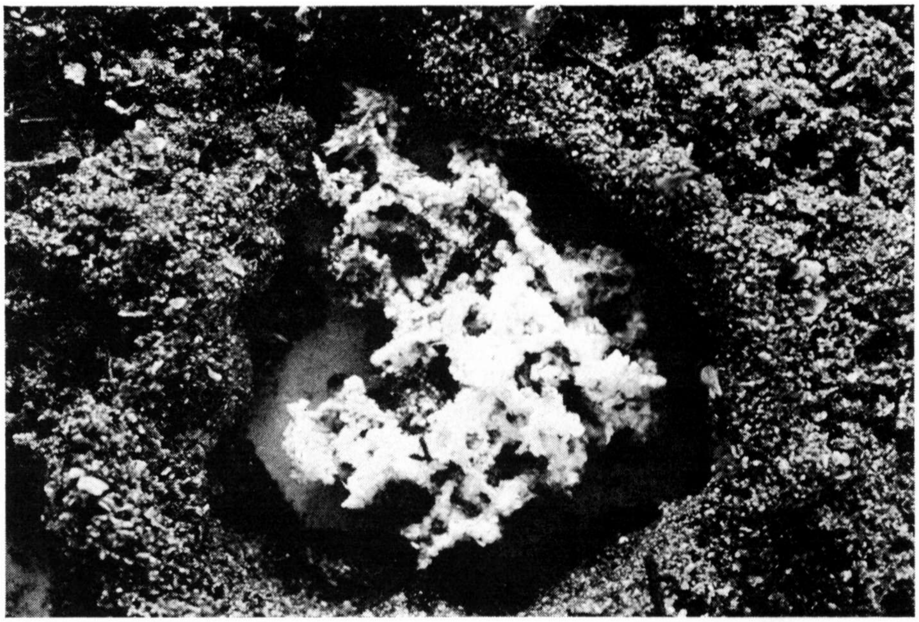
Fig. 23 (center). Fungus garden of *Apterostigma dentigerum* Wheeler under a rotted log in rain forest, Panama. The garden, covered with a thin mycelial veil, has a single opening for the ants, at left. Forceps, 113 millimeters long.

Fig. 24 (bottom). The garden of Fig. 23 with veil torn aside to show the loose cells of insect excrement covered with the ant fungus.

on the brood and on inert material like insect carcasses (*19, 21, 29, 37*). The brood, like the garden, is continually licked by the ants.

It appears probable that the products of the salivary glands and the anal droplets are of primary importance in creating the conditions described (*29, 43, 44*) (Fig. 27). These are consistently applied to the garden, in all species. The ants, in grooming, apply saliva copiously enough to keep the queen glistening (Fig. 28), and much of the time the workers are grooming one another. The combination of the two, or perhaps the products of the abdomen alone, are sufficiently pungent to give off an odor easily perceptible to blunted human senses. The odor of an *Acromyrmex lobicornis* colony in a confined observation nest was strongly ammoniacal. Species of *Atta* and *Acromyrmex octospinosus* have an entirely different odor. Ants preserved in ethanol for years will still give off the odor.

Some books containing chapters on the attines have errors that do not destroy their usefulness (see, for example, *45*). One of the chief myths is that the ant fungus does not develop the clusters of inflated hyphae (Fig. 13, *a, g*) in the absence of the ants. These clusters are produced in artificial culture containing no ants as well as in the absence of ants in nature (*18, 29, 43*). Wheeler suffered much abuse for casting doubt about Moeller's certainty that he had the true ant fungus in culture. Moeller was probably correct. He was cautious and his work was admirably thorough. One hopes that, after more than 70 years, his work in southern Brazil can be duplicated, or—bet-



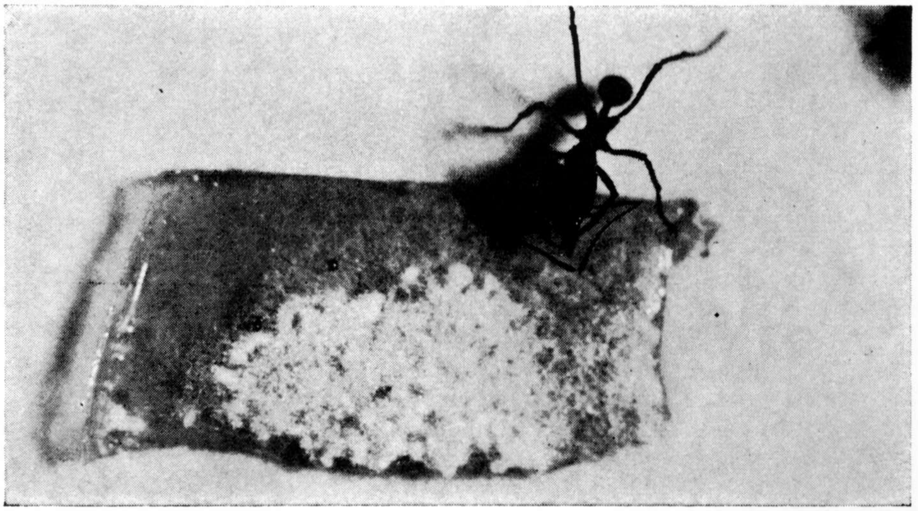


Fig. 25 (top). Fungus garden of *Mycetophylax conformis* after development on cassava granules. On the seashore, grass stems were generally used; these were retrieved from high tide level.

Fig. 26 (center). Fungus-garden fragment of a *Trachymyrmex septentrionalis* colony on nutrient agar in a Petri dish. Numerous bacterial and alien fungal colonies dot the agar, except in the vicinity of the garden. Here the ants (not shown) have cut the agar and piled the pieces at the side. These fragments, which have received some saliva in the cutting, do not grow alien organisms as fast as the untreated agar does and appear clean and shiny in contrast to adjacent contaminated agar surfaces.

Fig. 27 (bottom). Response of a worker of *Atta sexdens* to a culture of the fungus of *Trachymyrmex septentrionalis*. Fecal droplets (left) were soon deposited.

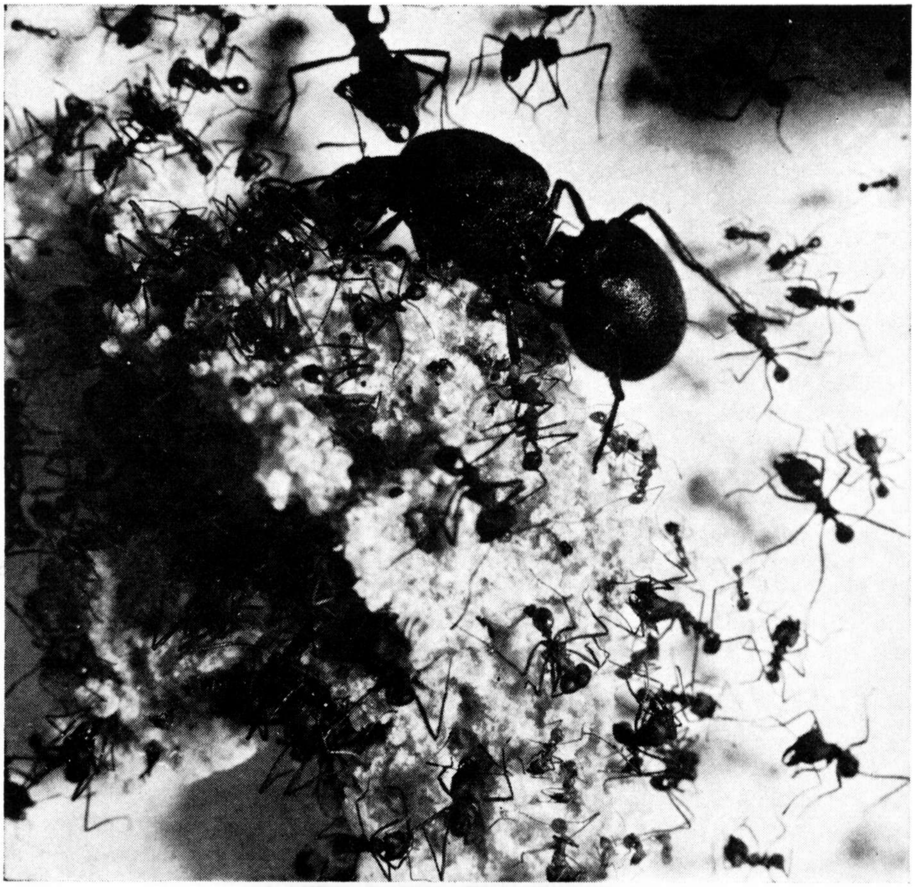
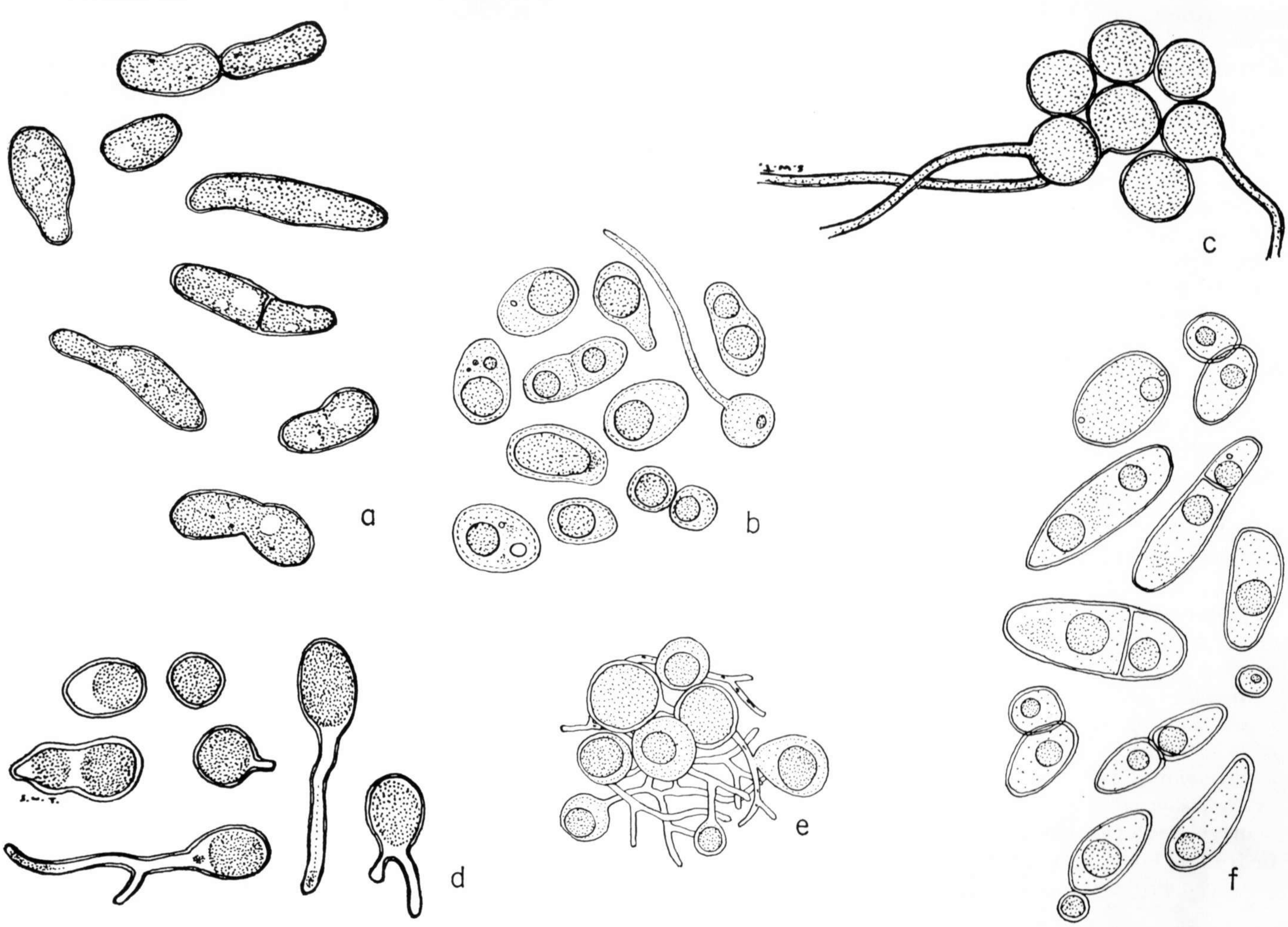


Fig. 28 (left). Queen of a young *Atta sexdens* colony on her fungus garden. The workers have kept her integument well licked. Panama.

Fig. 29 (below). The fungus of *Cyphomyrmex rimosus* Spinola growing as a yeast in the garden and as a mycelial growth on the brood or in artificial culture. The smallest cells are 8 microns in diameter, and hyphal projections are 2 to 3 microns wide. (*a*) Typical yeast form in a bromatium of the ant garden. (*b*) Yeast cells, one extending a hypha. (*c*) A cluster of seven cells from an ant larva skin, three of them extending hyphae. (*d*) Hyphae growing and forking from yeast cells. (*e*) Compact mass of cells and hyphae growing on the skin of an ant larva. (*f*) Yeast cells growing on cassava granules in an active ant observation nest.



ter yet—his sporophores can be produced in artificial culture from the stage present in the normal nest. Forel referred to ants sowing spores; if spores are produced the ants never sow them but abandon that part of the nest. Forel's statement that indigenous vegetation (as contrasted with exotics), "gradually reinforced by natural selection, resists the attacks" of the ants does not correspond with the situation. His dismissal, as a myth, of the story of carpets of green leaf sections in garden chambers was premature. Such carpets may be found after times of abundant leaf-cutting.

The Fungus

Fungi associated with attine ants have been given a number of names since Moeller described the first one as *Rozites gonglylophora* in 1893. A review appeared in 1938 (*46*). These fungi included *Xylaria, Bargellinia, Rhizomorpha, Locellinia, Poroniopsis, Lentinus*, and *Tyridiomyces* (Fig. 29) species belonging to the Ascomycetes, Basidiomycetes, and Fungi Imperfecti.

The assumption by investigators (*13, 18*) that the fungus grown by other *Acromyrmex* and *Atta* was the same as Moeller's *Rozites gonglylophora* of *Acromyrmex disciger* was challenged by Jacoby (*22*). The fungi are superficially much alike, as cultured by the ants, but Jacoby's experiments in which he exchanged gardens of the two fungus species resulted in rejection of each. I had performed this type of experiment earlier, in 1934–35 (*19, 37*), with many Trinidad species, and later with species of other countries. Where exchange of fungus gardens is attempted, a complication is the difference in pheromone substances of the two ant species, which the ants had added to the garden before the exchange; this difference may play a part in rejection. This complication is eliminated by using artificial cultures of the fungi (*19, 21, 29, 36, 41, 42*). Studies involving isolation of other fungi from laboratory colonies should be carried out (*27*). Rarely, conidial forms may develop (Fig. 13, *e* and *f*).

Moeller's *Rozites* has been reexamined taxonomically in recent years and is now referred to as *Leucocoprinus gongylophora* (*47*) or *Leucoagaricus* or *Agaricus gongylophora* (*48*). Meanwhile I developed the first sporophore or fruiting body of an ant fungus to be produced in the laboratory, by starting with the stage cultured by the ants (*29*) (Fig. 13*c*). This was considered to be a new species of *Lepiota* by Locquin. In 1965 W. J. Robbins and his associates produced what is apparently the same species of *Lepiota* but from a culture from another ant species, which I submitted to him. Heim (*47*) considered the first *Lepiota* to be the same as Moeller's. I believe this to be unlikely. If they were the same, we would have one species of fungus cultured by *Acromyrmex discigera* in south Brazil, by *Cyphomyrmex costatus* in Panama, and by *Myrmicocrypta buenzlii* in Trinidad. It is more likely that Moeller's species of fungus belongs to a group of similar species that are cultured by *Acromyrmex* and *Atta*. My tests of fungus cultures of a number of *Lepiota* species submitted by Robbins showed acceptances for eating (not culturing) by several attine species (*49*).

The other fungus genera of the 1938 review may be dismissed as follows.

The *Poroniopsis bruchi* Spegazzini from discarded substrate of *Acromyrmex (Moellerius) heyeri* and *Atta vollenweideri* is the same as *Hypocreodendron sanguineum* P. Henning (*50*). It has not been proved to be a true ant fungus. Another synonym is *Rhizomorpha formicarum* Spegazzini (loc. cit.). The new name *Discoxylaria mirmecophila* Lindquist and Wright applies (*51*).

The *Locellinia Mazzuchii* Spegazzini from a nest of *Atta vollenweideri* is considered to be a species of *Agaricus* (*47*). It is in the same category as *Lentinus atticolus* Weber 1938, not proved to be a true ant fungus. In both cases large mushrooms or sporophores were growing over ant nests. The fact that hyphae grew down to abandoned fungus gardens is not conclusive evidence that the fungus cultured by the ants was the same.

The *Xylaria micrura* of Spegazzini and, later, of Bruch is still not proved to be an ant fungus.

The *Tyridiomyces formicarum* Wheeler 1907 is still classified as a member of the Fungi Imperfecti since no sexual stage has been produced. It is the yeast cultured by *Cyphomyrmex rimosus* Spinola (Fig. 29). The hyphal stage shown in Fig. 29 is new and hitherto undescribed.

Additional ant fungi that I have developed from new sources are as follows.

1) The fungus cultured from a 1957 Panamanian colony of *Apterostigma mayri* Forel. A fructification developed in an oak flask culture; the culture was examined by Lekh Batra and submitted to G. W. Martin. The latter reported the fructification to be an *Auricularia*, perhaps *A. polytricha* (Mont.) Sacc., but, since it did not mature sufficiently, no further identification was possible.

2) The fungus cultured from a 1957 Panamanian colony of *Myrmicocrypta ednaella* Mann. A fructification developed in an oat flask culture but did not mature sufficiently for identification.

3) The fungus cultured from a 1957 Panamanian colony of *Cyphomyrmex rimosus* Spinola. Massive black sclerotia developed in a wheat flask culture; they were examined by W. C. Denison and Lekh Batra. The sclerotia were then sent to G. W. Martin, who characterized the subsequent culture and growth as that of a *Xylaria* with early similarities to *Daldinia*. The three mycologists agreed on its Ascomycetous nature.

In summary, the names presently applicable to ant fungi are as follows.

1) *Leucocoprinus, Leucoagaricus* or *Agaricus gongylophora* (Moeller 1893). Host ants: *Acromyrmex disciger*, possibly *Atta*.

2) *Tyridomyces formicarum* Wheeler 1907 (possibly a *Daldinia* or *Xylaria*). Host ant: *Cyphomyrmex rimosus*.

3) *Lepiota* n. sp. (Weber 1957, Robins 1965. Host ants: *Cyphomyrmex costatus, Myrmicocrypta buenzlii*.

4) *Auricularia* sp. Host ant: *Apterostigma mayri*.

Summary

Fungus-growing ants (Attini) are in reality unique fungus-culturing insects. There are several hundred species in some dozen genera, of which *Acromyrmex* and *Atta* are the conspicuous leaf-cutters. The center of their activities is the fungus garden, which is also the site of the queen and brood. The garden, in most species, is made from fresh green leaves or other vegetal material. The ants forage for this, forming distinct trails to the vegetation that is being harvested. The cut leaves or other substrate are brought into the nest and prepared for the fungus. Fresh leaves and flowers are cut into pieces a millimeter or two in diameter; the ants form them into a pulpy mass by pinching them with the mandibles and adding saliva. Anal droplets are deposited on

the pieces, which are then forced into place in the garden. Planting of the fungus is accomplished by an ant's picking up tufts of the adjacent mycelium and dotting the surface of the new substrate with it. The combination of salivary and anal secretions, together with the constant care given by the ants, facilitates the growth of the ant fungus only, despite constant possibilities for contamination. When the ants are removed, alien fungi and other organisms flourish.

A mature nest of *Atta sexdens* may consist of 2000 chambers, some temporarily empty, some with refuse, and the remainder with fungus gardens. Thousands of kilograms of fresh leaves will have been used. A young laboratory colony of *Atta cephalotes* will use 1 kilogram of fresh leaves for one garden. The attines are the chief agents for introducing organic matter into the soil in tropical rain forests; this matter becomes the nucleus for a host of other organisms, including nematodes and arthropods, after it is discarded by the ants.

One ant species cultures a yeast; all others grow a mycelium. In the higher species the mycelium forms clusters of inflated hyphae. Mycologists accept as valid two names for confirmed fruiting stages: *Leucocoprinus* (or *Leucoagaricus*) *gongylophora* (Moeller 1893) and *Lepiota* n. sp.

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